



## SEQUENCE LISTING

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<120> TAQ DNA POLYMERASES HAVING AN AMINO ACID SUBSTITUTION AT E681 AND  
HOMOLOGS THEREOF EXHIBITING IMPROVED SALT TOLERANCE

<130> PB9944

<140> US 10/049,358

<141> 2002-05-17

<150> PCT/US00/22150

<151> 2000-08-10

<150> US 60/154,739

<151> 1999-09-17

<150> US 60/150,167

<151> 1999-08-21

<160> 5

<170> PatentIn version 3.3

<210> 1

<211> 832

<212> PRT

<213> Thermus aquaticus

<400> 1

Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu  
1 5 10 15

Val Asp Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly  
20 25 30

Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala  
35 40 45

Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val  
50 55 60

Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly  
65 70 75 80

Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu  
85 90 95

Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu  
100 105 110

Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys  
 115 120 125  
 Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp  
 130 135 140  
 Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly  
 145 150 155 160  
 Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro  
 165 170 175  
 Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn  
 180 185 190  
 Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu  
 195 200 205  
 Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu  
 210 215 220  
 Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys  
 225 230 235 240  
 Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val  
 245 250 255  
 Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Phe  
 260 265 270  
 Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu Leu  
 275 280 285  
 Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly  
 290 295 300  
 Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp  
 305 310 315 320  
 Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro  
 325 330 335  
 Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu  
 340 345 350  
 Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro  
 355 360 365  
 Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn

370

375

380

Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu  
385 390 395 400

Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu  
405 410 415

Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu  
420 425 430

Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly  
435 440 445

Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala  
450 455 460

Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His  
465 470 475 480

Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe Asp  
485 490 495

Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys Arg  
500 505 510

Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro Ile  
515 520 525

Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser Thr  
530 535 540

Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg Leu  
545 550 555 560

His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser Ser  
565 570 575

Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly Gln  
580 585 590

Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val Ala  
595 600 605

Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser Gly  
610 615 620

Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His Thr  
625 630 635 640

Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp Pro  
645 650 655

Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Phe Gly Val Leu Tyr Gly  
660 665 670

Met Ser Ala His Arg Leu Ser Gln Glu Leu Ala Ile Pro Tyr Glu Glu  
675 680 685

Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val Arg  
690 695 700

Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr Val  
705 710 715 720

Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala Arg  
725 730 735

Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro  
740 745 750

Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu  
755 760 765

Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His  
770 775 780

Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala  
785 790 795 800

Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro  
805 810 815

Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu  
820 825 830

<210> 2

<211> 561

<212> PRT

<213> Thermus aquaticus

<400> 2

Met Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu  
1 5 10 15

Leu Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu  
20 25 30

Gly Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala  
 35 40 45

Asp Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala  
 50 55 60

Pro Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu  
 65 70 75 80

Leu Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu  
 85 90 95

Pro Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser  
 100 105 110

Asn Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr  
 115 120 125

Glu Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn  
 130 135 140

Leu Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg  
 145 150 155 160

Glu Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr  
 165 170 175

Gly Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val  
 180 185 190

Ala Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly  
 195 200 205

His Pro Phe Asn Leu Asn Ser Arg Asp Gln Leu Glu Arg Val Leu Phe  
 210 215 220

Asp Glu Leu Gly Leu Pro Ala Ile Gly Lys Thr Glu Lys Thr Gly Lys  
 225 230 235 240

Arg Ser Thr Ser Ala Ala Val Leu Glu Ala Leu Arg Glu Ala His Pro  
 245 250 255

Ile Val Glu Lys Ile Leu Gln Tyr Arg Glu Leu Thr Lys Leu Lys Ser  
 260 265 270

Thr Tyr Ile Asp Pro Leu Pro Asp Leu Ile His Pro Arg Thr Gly Arg  
 275 280 285

Leu His Thr Arg Phe Asn Gln Thr Ala Thr Ala Thr Gly Arg Leu Ser

290

295

300

Ser Ser Asp Pro Asn Leu Gln Asn Ile Pro Val Arg Thr Pro Leu Gly  
 305 310 315 320

Gln Arg Ile Arg Arg Ala Phe Ile Ala Glu Glu Gly Trp Leu Leu Val  
 325 330 335

Ala Leu Asp Tyr Ser Gln Ile Glu Leu Arg Val Leu Ala His Leu Ser  
 340 345 350

Gly Asp Glu Asn Leu Ile Arg Val Phe Gln Glu Gly Arg Asp Ile His  
 355 360 365

Thr Glu Thr Ala Ser Trp Met Phe Gly Val Pro Arg Glu Ala Val Asp  
 370 375 380

Pro Leu Met Arg Arg Ala Ala Lys Thr Ile Asn Tyr Gly Val Leu Tyr  
 385 390 395 400

Gly Met Ser Ala His Arg Leu Ser Gln Arg Leu Ala Ile Pro Tyr Glu  
 405 410 415

Glu Ala Gln Ala Phe Ile Glu Arg Tyr Phe Gln Ser Phe Pro Lys Val  
 420 425 430

Arg Ala Trp Ile Glu Lys Thr Leu Glu Glu Gly Arg Arg Arg Gly Tyr  
 435 440 445

Val Glu Thr Leu Phe Gly Arg Arg Arg Tyr Val Pro Asp Leu Glu Ala  
 450 455 460

Arg Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met  
 465 470 475 480

Pro Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys  
 485 490 495

Leu Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val  
 500 505 510

His Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val  
 515 520 525

Ala Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val  
 530 535 540

Pro Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys  
 545 550 555 560

Glu

<210> 3  
<211> 832  
<212> PRT  
<213> *Thermus aquaticus*

<400> 3

Met Arg Gly Met Leu Pro Leu Phe Glu Pro Lys Gly Arg Val Leu Leu  
1 5 10 15

Val Ala Gly His His Leu Ala Tyr Arg Thr Phe His Ala Leu Lys Gly  
20 25 30

Leu Thr Thr Ser Arg Gly Glu Pro Val Gln Ala Val Tyr Gly Phe Ala  
35 40 45

Lys Ser Leu Leu Lys Ala Leu Lys Glu Asp Gly Asp Ala Val Ile Val  
50 55 60

Val Phe Asp Ala Lys Ala Pro Ser Phe Arg His Glu Ala Tyr Gly Gly  
65 70 75 80

Tyr Lys Ala Gly Arg Ala Pro Thr Pro Glu Asp Phe Pro Arg Gln Leu  
85 90 95

Ala Leu Ile Lys Glu Leu Val Asp Leu Leu Gly Leu Ala Arg Leu Glu  
100 105 110

Val Pro Gly Tyr Glu Ala Asp Asp Val Leu Ala Ser Leu Ala Lys Lys  
115 120 125

Ala Glu Lys Glu Gly Tyr Glu Val Arg Ile Leu Thr Ala Asp Lys Asp  
130 135 140

Leu Tyr Gln Leu Leu Ser Asp Arg Ile His Val Leu His Pro Glu Gly  
145 150 155 160

Tyr Leu Ile Thr Pro Ala Trp Leu Trp Glu Lys Tyr Gly Leu Arg Pro  
165 170 175

Asp Gln Trp Ala Asp Tyr Arg Ala Leu Thr Gly Asp Glu Ser Asp Asn  
180 185 190

Leu Pro Gly Val Lys Gly Ile Gly Glu Lys Thr Ala Arg Lys Leu Leu  
195 200 205

Glu Glu Trp Gly Ser Leu Glu Ala Leu Leu Lys Asn Leu Asp Arg Leu  
210 215 220

Lys Pro Ala Ile Arg Glu Lys Ile Leu Ala His Met Asp Asp Leu Lys  
225 230 235 240

Leu Ser Trp Asp Leu Ala Lys Val Arg Thr Asp Leu Pro Leu Glu Val  
245 250 255

Asp Phe Ala Lys Arg Arg Glu Pro Asp Arg Glu Arg Leu Arg Ala Phe  
260 265 270

Leu Glu Arg Leu Glu Phe Gly Ser Leu Leu His Glu Phe Gly Leu Leu  
275 280 285

Glu Ser Pro Lys Ala Leu Glu Glu Ala Pro Trp Pro Pro Pro Glu Gly  
290 295 300

Ala Phe Val Gly Phe Val Leu Ser Arg Lys Glu Pro Met Trp Ala Asp  
305 310 315 320

Leu Leu Ala Leu Ala Ala Ala Arg Gly Gly Arg Val His Arg Ala Pro  
325 330 335

Glu Pro Tyr Lys Ala Leu Arg Asp Leu Lys Glu Ala Arg Gly Leu Leu  
340 345 350

Ala Lys Asp Leu Ser Val Leu Ala Leu Arg Glu Gly Leu Gly Leu Pro  
355 360 365

Pro Gly Asp Asp Pro Met Leu Leu Ala Tyr Leu Leu Asp Pro Ser Asn  
370 375 380

Thr Thr Pro Glu Gly Val Ala Arg Arg Tyr Gly Gly Glu Trp Thr Glu  
385 390 395 400

Glu Ala Gly Glu Arg Ala Ala Leu Ser Glu Arg Leu Phe Ala Asn Leu  
405 410 415

Trp Gly Arg Leu Glu Gly Glu Glu Arg Leu Leu Trp Leu Tyr Arg Glu  
420 425 430

Val Glu Arg Pro Leu Ser Ala Val Leu Ala His Met Glu Ala Thr Gly  
435 440 445

Val Arg Leu Asp Val Ala Tyr Leu Arg Ala Leu Ser Leu Glu Val Ala  
450 455 460

Glu Glu Ile Ala Arg Leu Glu Ala Glu Val Phe Arg Leu Ala Gly His



465		470		475		480
Pro Phe Asn Leu	Asn Ser Arg Asp Gln	Leu Glu Arg Val	Leu Phe Asp			
	485		490		495	
Glu Leu Gly	Leu Pro Ala Ile Gly	Lys Thr Glu Lys Thr	Gly Lys Arg			
	500		505		510	
Ser Thr	Ser Ala Ala Val	Leu Glu Ala Leu Arg Glu	Ala His Pro Ile			
	515		520		525	
Val Glu Lys Ile	Leu Gln Tyr Arg Glu Leu Thr	Lys Leu Lys Ser Thr				
	530		535		540	
Tyr Ile Asp Pro	Leu Pro Asp Leu Ile His	Pro Arg Thr Gly Arg	Leu			
	550		555		560	
His Thr Arg Phe	Asn Gln Thr Ala Thr	Ala Thr Gly Arg Leu	Ser Ser			
	565		570		575	
Ser Asp Pro	Asn Leu Gln Asn Ile	Pro Val Arg Thr Pro	Leu Gly Gln			
	580		585		590	
Arg Ile Arg	Arg Ala Phe Ile	Ala Glu Glu Gly Trp	Leu Leu Val Ala			
	595		600		605	
Leu Asp Tyr	Ser Gln Ile Glu	Leu Arg Val Leu	Ala His Leu Ser Gly			
	610		615		620	
Asp Glu Asn Leu	Ile Arg Val Phe Gln Glu	Gly Arg Asp Ile His	Thr			
	625		630		635	
Glu Thr Ala Ser	Trp Met Phe Gly Val	Pro Arg Glu Ala Val	Asp Pro			
	645		650		655	
Leu Met Arg	Arg Ala Ala Lys Thr	Ile Asn Tyr Gly Val	Leu Tyr Gly			
	660		665		670	
Met Ser	Ala His Arg Leu Ser	Gln Arg Leu Ala Ile	Pro Tyr Glu Glu			
	675		680		685	
Ala Gln Ala Phe	Ile Glu Arg Tyr Phe Gln Ser	Phe Pro Lys Val Arg				
	690		695		700	
Ala Trp Ile Glu	Lys Thr Leu Glu Glu Gly	Arg Arg Arg Gly Tyr Val				
	705		710		715	
Glu Thr Leu Phe	Gly Arg Arg Arg Tyr	Val Pro Asp Leu Glu	Ala Arg			
	725		730		735	

Val Lys Ser Val Arg Glu Ala Ala Glu Arg Met Ala Phe Asn Met Pro  
740 745 750

Val Gln Gly Thr Ala Ala Asp Leu Met Lys Leu Ala Met Val Lys Leu  
755 760 765

Phe Pro Arg Leu Glu Glu Met Gly Ala Arg Met Leu Leu Gln Val His  
770 775 780

Asp Glu Leu Val Leu Glu Ala Pro Lys Glu Arg Ala Glu Ala Val Ala  
785 790 795 800

Arg Leu Ala Lys Glu Val Met Glu Gly Val Tyr Pro Leu Ala Val Pro  
805 810 815

Leu Glu Val Glu Val Gly Ile Gly Glu Asp Trp Leu Ser Ala Lys Glu  
820 825 830

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<213> Artificial Sequence

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<213> Artificial Sequence

<220>  
<223> Synthetic Oligonucleotide

<400> 5  
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